Please amend the subject application as follows.

In the Title:

In accordance with 37 C.F.R. §§1.121(b)(1)(i)-(ii), please delete the title beginning on page 1 of the application and replace it with the following:

-- METHODS FOR GENERATING AND IDENTIFYING ANTIBODIES DIRECTED AGAINST A B7 --

In the Specification:

Please the amend the specification as follows:

Please replace the paragraph beginning at page 9, lines 21-25, with the following rewritten paragraph:

-- Figure 7a is a graph showing the effects of DR7-primed $CD4^+CD45RO^+$ T_h cells on differentiation of B cells into IgM secreting SKW B cells, as described in Example 2, *infra*.

Figure 7b is a graph showing the effects of DR7-primed $CD4^+CD45RO^+$ T_h cells on differentiation of B cells into IgG secreting CESS B cells, as described in Example 2, *infra*.--

Please replace the paragraph beginning at page 9, lines 28-30, with the following rewritten paragraph:

-- Figure 8a is a graph showing the effect of anti-CD28 and anti-B7 mAbs on the T_h-induced production of IgM by B cells as described in Example 2, *infra*.

Figure 8a is a graph showing the effect of anti-CD28 and anti-B7 mAbs on the T_h-induced production of IgG by B cells as described in Example 2, *infra*.--

Please replace the paragraph beginning at page 9, lines 32-35, with the following rewritten paragraph:

-- Figure 9a is a diagrammatic representation of B7Ig protein fusion constructs as described in Example 3, *infra* (dark shaded regions = oncostatin M; unshaded regions = B7, stippled regions = human Ig Cγ1).

Figure 9b is a diagrammatic representation of CD28Ig protein fusion constructs as described in Example 3, *infra* (dark shaded regions = oncostatin M; unshaded regions = CD28, stippled regions = human Ig Cγ1).--

Please replace the paragraph beginning at page 11, line 8, with the following rewritten paragraph:

-- Recently, Freeman et al., (J. Immunol. 143 (8): 2714-2722 (1989)) isolated and sequenced a cDNA clone encoding a B cell activation antigen recognized by monoclonal antibody (mAb) B7 (Freedman et al., J. Immunol. 139:3260 (1987)). COS cells transfected with this cDNA were shown to stain by both mAb B7 and mAb BB-1 (Clark et al., Human Immunology 16:100-113 (1986), and Yokochi et al., (1981), supra; Freeman et al., (1989) supra; and Freedman et al., (1987), supra)). The ligand for CD28 was identified by the experiments described herein, as the B7/BB-1 antigen isolated by Freeman et al., wherein the predicted amino acid sequence of amino acid 1-216 are:

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Gly Leu Ser His Phe Cys Ser Gly Val Ile His Val Thr Lys Glu Val
  1               5                  10                  15

Lys Glu Val Ala Thr Leu Ser Cys Gly His Asn Val Ser Val Glu Glu
             20                  25                  30

Leu Ala Gln Thr Arg Ile Tyr Trp Gln Lys Glu Lys Lys Met Val Leu
         35                  40                  45

Thr Met Met Ser Gly Asp Met Asn Ile Trp Pro Glu Tyr Lys Asn Arg
     50                  55                  60

Thr Ile Phe Asp Ile Thr Asn Asn Leu Ser Ile Val Ile Leu Ala Leu
 65                  70                  75                  80

Arg Pro Ser Asp Glu Gly Thr Tyr Glu Cys Val Val Leu Lys Tyr Glu
                 85                  90                  95

Lys Asp Ala Phe Lys Arg Glu His Leu Ala Glu Val Thr Leu Ser Val
            100                 105                 110

Lys Ala Asp Phe Pro Thr Pro Ser Ile Ser Asp Phe Glu Ile Pro Thr
        115                 120                 125

Ser Asn Ile Arg Arg Ile Ile Cys Ser Thr Ser Gly Gly Phe Pro Glu
    130                 135                 140

Pro His Leu Ser Trp Leu Glu Asn Gly Glu Glu Leu Asn Ala Ile Asn
145                 150                 155                 160

Thr Thr Val Ser Gln Asp Pro Glu Thr Glu Leu Tyr Ala Val Ser Ser
                165                 170                 175

Lys Leu Asp Phe Asn Met Thr Thr Asn His Ser Phe Met Cys Leu Ile
            180                 185                 190

Lys Tyr Gly His Leu Arg Val Asn Gln Thr Phe Asn Trp Asn Thr Thr
        195                 200                 205

Lys Gln Glu His Phe Pro Asp Asn
    210                 215
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(Freedman et al., and Freeman et al., supra, both of which are incorporated by reference herein).--

On page 13, please replace the first full paragraph with:

-- In a preferred embodiment, DNA encoding the amino acid sequence corresponding to the extracellular domain of the B7 antigen, containing amino acids from about position 1 to about position 215, is joined to DNA encoding the amino acid sequences

corresponding to the hinge, CH2 and CH3 regions of human Ig Cγ1, using PCR, to form a construct that is expressed as B7Ig fusion protein. DNA encoding the amino acid sequence corresponding to the B7Ig fusion protein has been deposited with the American Type Culture Collection (ATCC) at 10801 University Blvd., Manassas, Virginia 20110-2209 U.S.A., under the Budapest Treaty on May 31, 1991 and accorded accession number 68627.

On page 30, line 14, please replace the paragraph beginning "Cell Culture" with the following paragraph:

-- Cell Culture. T51, 1A2, 5E1, Daudi, Raji, Jijoye, CEM, Jurkat, HSB2, THP-1 and HL60 cells (Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA) were cultured in complete RPMI™ medium (RPMI™ containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin. Dhfr-deficient Chinese hamster ovary (CHO) cells (Urlaub and Chasin, Proc. Natl. Acad Sci., 77:4216-4220 (1980)) were cultured in Maintenance Medium (Ham's F12 Medium™ (GIBCO, Grand Island, NY) supplemented with 10% FBS, 0.15 mM L-proline, 100 U/ml penicillin and 100 μg/ml streptomycin). Dhfr-positive transfectants were selected and cultured in Selective Medium (DMEM™, supplemented with 10% FBS, 0.15 mM L-proline, 100 U/ml penicillin and 100 μg/ml streptomycin).--

On page 30, line 28, please replace the paragraph bridging pages 30 and 31, and beginning "Spleen B cells", with the following paragraph:

-- Spleen B cells were purified from Balb/c mice by treatment of total spleen cells with an anti-Thy 1.2 mAb (30H12) (Ledbetter and Herzenberg, Immunol. Rev. 47:361-389 (1979)) and baby rabbit complement. The resulting preparations contained approximately 85% B cells, as judged by FACS® analysis following staining with fluorescein isothiothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin (TAGO). These

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cells were activated by treatment for 72 hrs with E. coli lipopolysaccharide (LPS, List Biological Laboratories, Campbell, CA) at 10 μg/ml in complete RPMI™.--

On page 31, line 5, please replace the paragraph "Monoclonal Antibodies" with the following:

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-- Monoclonal Antibodies. Monoclonal antibody (mAb) 9.3 (anti-CD28) (ATCC No. HB 10271, Hansen et al., Immunogenetics 10:247-260 (1980)) was purified from ascites before use. mAb 9.3 $F(ab')_2$ fragments were prepared as described by Parham, in J. Immunol. 131:2895-2902 (1983). Briefly, purified mAb 9.3 was digested with pepsin at pH 4.1 for 75 min. followed by passage over protein A SEPHAROSE™ (beaded agarose) to remove undigested mAb. A number of mAbs to B cell-associated antigens were screened for their abilities to inhibit CD28-mediated adhesion. mAbs 60.3 (CD18); 1F5 (CD20); G29-5 (CD21); G28-7, HD39, and HD6 (CD22); HD50 (CD23); KB61 (CD32); G28-1 (CD37); G28-10 (CD39); G28-5 (CD40); HERMES1 (CD44); 9.4 (CD45); LB-2 (CD54) and 72F3 (CD71) have been previously described and characterized in International Conferences on Human Leukocyte Differentiation Antigens I-III (Bernard et al., Eds., Leukocyte Typing, Springer-Verlag, New York (1984); Reinherz et al., Eds., Leukocyte Typing II Vol. 2 New York (1986); and McMichael et al., Eds., Leukocyte Typing III Oxford Univ. Press, New York, (1987)). These mAbs were purified before use by protein A SEPHAROSE™ (beaded agarose) chromatography or by salt precipitation and in exchange chromatography. δTA401 (Kuritani and Cooper, J. Exp. Med. 155:839-848 (1982)) (Anti-IgD); 2C3 (Clark et al., (1986), supra) (anti-IgM); Namb1, H1DE, P10.1, W6/32 (Clark et al., (1986) supra; and Gilliland et al., Human Immunology 25:269-289 (1989), anti-human class I); and HB10A (Clark et al., (1986), supra, anti-MHC class II) were also purified before use. mAbs B43 (CD19); BL-40 (CD72); AD2, 1E9.28.1, and 7G2.2.11 (CD73); EBU-141, LN1 (CDw75); CRIS-1 (CD-76); 424/4A11, 424/3D9 (CD77) Leu 21, Ba, 1588, LO-panB-1, FN1, and FN4 (CDw78); and M9, G28-10, HuLym10, 2-7, F2B2.6, 121, L26, HD77, NU-B1, BLAST-1, BB-1, anti-BL7, anti-

HC2, and L23 were used as coded samples provided to participants in the Fourth International Conference on Human Leukocyte Differentiation Antigens (Knapp, Ed., Leukocyte Typing IV, Oxford Univ. Press, New York (1990). These were used in ascites form. mAbs BB-1 and LB-1 (Yokochi et al., (1981), supra) were also purified from ascites before use. Anti-integrin receptor mAbs P3E3, P4C2, P4G9 (Wayner et al., J. Cell. Biol. 109:1321-1330 (1989)) were used as hybridoma culture supernatants.--

On page 32, line 10, please replace the paragraph beginning "Immunostaining Techniques." with the following:

-- Immunostaining Techniques. For indirect immunofluorescence, cells were incubated with mAbs at 10 μg/ml in complete RPMI™ for 1 hr at 4°C. mAb binding was detected with a FITC-conjugated goat anti-mouse immunoglobulin second step reagent. For direct binding experiments, mAbs 9.3 and BB-1 were directly conjugated with FITC as described by Goding in Monoclonal Antibodies: Principles and Practices Academic Press, Orlando, FL (1983), and were added at saturating concentrations in complete RPMI™ for 1 hr at 4°C. Non-specific binding of FITC-conjugated mAbs was measured by adding the FITC conjugate following antigen pre-blocking (20-30 min at 4°C) with unlabeled mAb9.3 or BB-1. Immunohistological detection of adherent lymphoblastoid cells was achieved using the horseradish peroxidase (HRP) method described by Hellstrom et al., J. Immunol. 127:157-160 (1981).--

On page 34, line 15, please replace the two paragraphs beginning "CD28-Mediated Adhesion Assay" and "Labeled cells" with the following two paragraphs:

-- CD28-Mediated Adhesion Assay. Cells to be tested for adhesion were labeled with ^{51}Cr (0.2-1 mCi) to specific activities of 0.2-2 cpm/cell. A mouse mAb having irrelevant specificity, mAb W1, directed against human breast carcinoma-associated mucin, (Linsley et al., Cancer Res. 46:5444-5450 (1986)), was added to the labeling reaction to a

final concentration of 100 μg/ml to saturate Fc receptors. Labeled and washed cells were preincubated in complete RPMI™ containing 10 μg/ml of mAb W1, and unless otherwise indicated, 10 mM EDTA. mAb 9.3 or mAb 9.3 F(ab')$_2$ was added to some samples at 10 μg/ml, for approximately 1 hr at 23°C.

Labeled cells (1-10 x 10^6/well in a volume of 0.2 ml complete RPMI ™, containing EDTA and mAbs, where indicated) were then added to the CHO monolayers. Adhesion was initiated by centrifugation in a plate carrier (1,000 rpm, in a Sorvall HB1000 rotor, approximately 210 X g) for 3 min at 4°C. Plates were then incubated at 37°C for 1 hr. Reactions were terminated by aspirating unbound cells and washing five times with cold, complete RPMI™. Monolayers were solubilized by addition of 0.5 N NaOH, and radioactivity was measured in a gamma counter. For most experiments, numbers of bound cells were calculated by dividing total bound radioactivity (cpm) by the specific activity (cpm/cell) of labeled cells. When COS cells were used, their viability at the end of the experiment was generally less than 50%, so specific activity calculations were less accurate. Therefore, for COS cells results are expressed as cpm bound.--

On page 35, line 22, please replace the paragraph beginning "The effects of divalent cation depletion" with the following:

-- The effects of divalent cation depletion on T51 cell adhesion to $CD28^+$ and $CD28^-$ CHO cells were examined. Preliminary experiments showed that EDTA treatment caused loss of CHO cells during washing, so the CHO cell monolayers were fixed with paraformaldehyde prior to EDTA treatment. Fixation did not significantly affect CD28-mediated adhesion by T51 cells either in the presence or absence of mAb 9.3. Monolayers of $CD28^+$ and $CD28^-$ CHO cells (1 to 1.2 X 10^5/cm^2 in 48 well plastic dishes) were fixed in 0.5% paraformaldehyde for 20 min at 23°C, washed and blocked in Complete RPMI™ for 1 hr, then pre-incubated with or without mAb 9.3 or mAb 9.3 F(ab')$_2$ at 10 μg/ml in Complete RPMI ™ for 1 hr at 37°C. T51 cells were labeled with

^{51}Cr, preincubated with or without 10mM EDTA, added to CHO cells and cellular adhesion was measured. The results are presented in Figure 1. Mean and standard deviation (error bars) are shown for three replicate determinations.--

On page 45, line 9, please replace the paragraph beginning "Culture medium" with the following:

-- Culture medium. Complete culture medium (CM) consisted of RPMI™ 1640 (Irvine Scientific, Santa Ana, CA) supplemented with 100 U/ml of penicillin G, 100 μg/ml of streptomycin, 2mM L-glutamine, 5 X 10^{-5} M 2-ME, and 10% FBS (Irvine Scientific).--

On page 55, line 31 through page 56, line 30, please replace the two paragraphs beginning "Cell Culture and Transfections" and "CHO cells expressing CD28," with the following paragraphs:

-- Cell Culture and Transfections. COS (monkey kidney cells) were transfected with expression plasmids using a modification of the protocol of Seed and Aruffo (Proc. Natl. Acad. Sci. 84:3365 (1987)), incorporated by reference herein. Cells were seeded at 10^6 per 10 cm diameter culture dish 18-24 h before transfection. Plasmid DNA was added (approximately 15 μg/dish) in a volume of 5 ml of serum-free DMEM™ containing 0.1 mM cloroquine and 600 μg/ml DEAE Dextran™, and cells were incubated for 3-3.5 h at 37°C. Transfected cells were then briefly treated (approximately 2 min) with 10% dimethyl sulfoxide in PBS and incubated at 37°C for 16-24 h in DMEM™ containing 10% FCS. At 24 h after transfection, culture medium was removed and replaced with serum-free DMEM™ (6 ml/dish). Incubation was continued for 3 days at 37 °C, at which time the spent medium was collected and fresh serum-free medium was added. After an additional 3 days at 37°C, the spent medium was again collected and cells were discarded.

CHO cells expressing CD28, CD5 or B7 were isolated as described by Linsley et al., (1991) supra, as follows: Briefly, stable transfectants expressing CD28, CD5, or B7, were isolated following cotransfection of dihydrofolate reductase-deficient Chinese hamster ovary ($dhfr^-$ CHO) cells with a mixture of the appropriate expression plasmid and the selectable marker, pSV2dhfr, as described above in Example 1. Transfectants were then grown in increasing concentrations of methotrexate to a final level of 1 μM and were maintained in DMEM™ supplemented with 10% fetal bovine serum (FBS), 0.2 mM proline and 1 μM methotrexate. CHO lines expressing high levels of CD28 ($CD28^+$ CHO) or B7 ($B7^+$ CHO) were isolated by multiple rounds of fluorescence-activated cell sorting ($FACS^R$) following indirect immunostaining with mAbs 9.3 or BB-1. Amplified CHO cells negative for surface expression of CD28 or B7 ($dhfr^+$ CHO) were also isolated by $FACS^R$ from CD28-transfected populations. --

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On page 56, line 33, please replace the paragraph beginning "Immunostaining and $FACS^R$ Analysis," and continuing on page 57, with the following:

-- Immunostaining and $FACS^R$ Analysis. Transfected CHO cells or activated T cells were analyzed by indirect immunostaining. Before staining, CHO cells were removed from their culture vessels by incubation in PBS containing 10 mM EDTA. Cells were first incubated with murine mAbs 9.3 (Hansen et al., Immunogenetics 10:247 (1980)) or BB-1 (Yokochi et al., supra) at 10 μg/ml, or with Ig fusion proteins (CD28Ig, B7Ig, CD5Ig or chimeric mAb L6 containing Ig Cγ1, all at 10 μg/ml in DMEM™ containing 10% FCS) for 1-2 h at 4°C. Cells were then washed and incubated for an additional 0.5-2h at 4°C with FITC-conjugated second step reagent (goat anti-mouse Ig serum for murine mAbs, or goat anti-human Ig Cγ serum for fusion proteins (Tago, Inc., Burlingame, CA). Fluorescence was analyzed on 10,000 stained cells using a FACS IV^R cell sorter (Becton Dickinson and Co., Mountain View, CA) equipped with a four decade logarithmic amplifier.--

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On page 58, line 1, please replace the paragraph beginning "SDS Page" with the following:

-- SDS Page. SDS-PAGE was performed on linear acrylamide gradients gels with stacking gels of acrylamide. Aliquots (1 μg) B7Ig (lanes 1 and 3 of Figure 10) or CD28Ig (lanes 2 and 4) were subjected to SDS-PAGE (4-12% acrylamide gradient) under nonreducing (-βME, lanes 1 and 2) or reducing (+βME, lanes 3 and 4) conditions. Lane 5 of Figure 10 shows molecular weight (M_r) markers. Gels were stained with Coomassie Brilliant Blue, destained, and photographed or dried and exposed to X-ray film (Kodak™ XAR-5; Eastman Kodak Co., Rochester, NY) for autoradiography to visualize proteins.--

Please replace the three sequential paragraphs starting on page 59, line 27, with "Radiolabeling of B7Ig," and continuing to page 61, line 8, with the following three paragraphs:

-- Radiolabeling of B7Ig. Purified B7Ig (25μg) in a volume of 0.25 ml of 0.12 M sodium phosphate, pH 6.8 was iodinated using 2 mCi ^{125}I and 10 μg of chloramin T™. After 5 min at 23°C, the reaction was stopped by the addition of 20 μg sodium metabisulfite, followed by 3 mg of KI and 1 mg of BSA. Iodinated protein was separated from untreated ^{125}I by chromatography on a 5-ml column of Sephadex™ G-10 equilibrated with PBS containing 10% FCS. Peak fractions were collected and pooled. The specific activity of ^{125}I-B7Ig labeled in this fashion was 1.5 x 10^6 cpm/pmol.

B7Ig was also metabolically labeled with [^{35}S]methionine. COS cells were transfected with a plasmid encoding B7Ig as described above. At 24 h after transfection, [^{35}S]methionine (<800 Ci/mmol; Amersham Corp., Arlington Heights, IL) was added tc concentrations of 115 μCi/ml) in DMEM™ containing 10% FCS and 10% normal levels of methionine. After incubation at 37°C for 3 d, medium was collected and used for purification of B7Ig as described above. Concentrations of [^{35}S]methionine-labeled B7Ig

were estimated by comparison of staining intensity after SDS-PAGE with intensities of known amounts of unlabeled B7Ig. The specific activity of [^{35}S]methionine-labeled B7Ig was approximately 2 x 10^6 cpm/μg.

Binding Assays. For assays using immobilized CD28Ig, 96-well plastic dishes were coated for 16-24 h with a solution containing CD28Ig (0.5 μg in a volume of 0.05 ml of 10 mM Tris, pH 8). Wells were then blocked with binding buffer (DMEM™ containing 50 mM BES, pH 6.8, 0.1% BSA, and 10% FCS) (Sigma Chemical Co., St. Louis, MO) before addition of a solution (0.09 ml) containing ^{125}I-B7Ig (approximately 3 x 10^6 cpm, 2 X 10^6 cpm/pmol) or [^{35}S]-B7Ig (1.5 x 10^5 cpm) in the presence of absence of competitor to a concentration of 24 nM in the presence of the concentrations of unlabeled chimeric L6 mAb, mAb 9.3, mAb BB-1 or B7Ig, as indicated in Figure 12. After incubation for 2-3 h at 23°C, wells were washed once with binding buffer, and four times with PBS. Plate-bound radioactivity was then solubilized by addition of 0.5 N NaOH, and quantified by liquid scintillation or gamma counting. In Figure 12, radioactivity is expressed as a percentage of radioactivity bound to wells treated without competitor (7,800 cpm). Each point represents the mean of duplicate determinations; replicates generally varied from the mean by ≤ 20%. Concentrations were calculated based on M_r of 75,000 per binding site for mAbs and 51,000 per binding site for B7Ig. When binding of ^{125}I-B7 to CD28$^+$ CHO cells was measured, cells were seeded (2.5 x 10^4/well) in 96-well plates 16-24 h before the start of the experiment. Binding was otherwise measured as described above.--

On page 62, line 11, please replace the paragraph beginning "Cell separation and Stimulation" with the following:

-- Cell Separation and Stimulation. PBL were isolated by centrifugation through Lymphocyte Separation Medium™ (Litton Bionetics, Kensington, MD) and cultured in 96-well, flat-bottomed plates (4 x 10^4 cells/well, in a volume of 0.2 ml) in RPMI™ containing 10% FCS. Cellular proliferation of quadruplicate cultures was measured by

uptake of [^{3}H]thymidine during the last 5 h of a 3 day (d) culture. PHA-activated T cells were prepared by culturing PBL with 1 μg/ml PHA (Wellcome) for 5 d, and 1 d in medium lacking PHA. Viable cells were collected by sedimentation through Lymphocyte Separation Medium™ before use.--

On page 69, line 1, please replace the paragraph beginning "RNA was prepared" with the following:

-- RNA was prepared from stimulated PHA blasts by the procedure described by Chomczynki and Sacchi, Anal. Biochem. 162:156 (1987), incorporated by reference herein. Aliquots of RNA (20 μg) were fractionated on formaldehyde agarose gels and then transferred to nitrocellulose by capillary action. RNA was crosslinked to the membrane by UV light in a Stratalinker™ (Stratagene, San Diego, CA), and the blot was prehybridized and hybridized with a ^{32}P-labeled probe for human IL-2 (prepared from an approximately 600-bp cDNA fragment provided by Dr. S. Gillis; Immunex Corp., Seattle, WA). Equal loading of RNA samples was verified both by rRNA staining and by hybridization with a rat glyceraldehyde-6-phosphate dehydrogenase probe (GAPDH, an approximately 1.2-kb cDNA fragment provided by Dr. A. Purchio, Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA).--

At page 78, line 21, please add SEQ. ID. NO. 8 as submitted by paper and on the enclosed diskette.